PERPUSTAKAAN HAMDAN TAHIR
UNIVERSITI SAINS MALAYSIA

RUJUKAN

LAPORAN AKHIR
GERAN USM JANGKA PENDEK

A STUDY ON THE GJB3 AND GJB6 GENE MUTATIONS AMONG MALAYS WITH NON-SYNDROMIC HEARING LOSS

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UNIVERSITI SAINS MALAYSIA
PERPUSTAKAAN
08 DEC 2009
Perubatan

SENARAI SEMAKAN UNTUK BUKU LAPORAN AKHIR GERAN USM JANGKA PENDEK

NAMA PENYELIDIK UTAMA	:	**DR. MOHD. KHAIRI MD. DAUD**
NAMA CO-RESEARCHER	:	**DR. NORMASTURA ABD. RAHMAN, DR. ZAFARINA ZAINUDDIN**
TAJUK GERAN	:	**A study on the GJB3 and GJB6 gene mutations among Malays with non- syndromic hearing loss**
NO.AKAUN	:	**304/PPSP/6131446**

SENARAI SEMAKAN SEMASA PENYERAHAN BUKU LAPORAN AKHIR
(Sila Tandakan (4) Pada Kotak Yang Berkenaan)

NO.	PERKARA	ADA	TIADA
1.	Borang Laporan Akhir Projek Penyelidikan USM Jangka Pendek	/	
2.	Borang Laporan Hasil Penyelidikan, PPSP	/	
3.	i) Salinan Menuskrip	/	
	ii) Salinan surat/email bukti penghantaran kepada mana-mana journal		
4.	Penyata Perbelanjaan (Financial Statement) (Sila dapatkan daripada Jabatan Bendahari)	/	
5.	Laporan Komprehensif (termasuk kertas persidangan atau seminar dan penerbitan saintifik hasil daripada projek ini)	/	
6.	Surat pemakluman penghantaran Laporan Akhir ke Bhg. Penyelidikan	/	

Nota:

* Sila buat 3 salinan buku laporan Akhir
* No. 1-5 - Perlu dimasukkan dalam Buku Laporan Akhir
* No.6 - Hantar terus Kepada Pn. Che Merah Ismail (RCMO) hanya salinan kepada Bhg. R&D, PPSP

My doc/checklist borang2/sue

UNIVERSITI SAINS MALAYSIA

LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA *PENDEK*

FINAL REPORT OF SHORT TERM RESEARCH PROJECT

Sila kemukakan laporan akhir ini melalui Jawatankuasa Penyelidikan di Pusat Pengajian dan Dekan/Pengarah/Ketua Jabatan kepada Pejabat Pelantar Penyelidikan

1. Nama Ketua Penyelidik:
Name of Research Leader

☐ Profesor Madya/ *Assoc. Prof.* ☐ Dr./ *Dr.* ☐ Encik/Puan/Cik *Mr/Mrs/Ms*

2. Pusat Tanggungjawab (PTJ): *School/Department* Jabatan Otorhinolaringologi, Pusat Pengajian Sains Perubatan

3. Nama Penyelidik Bersama: *Name of Co-Researcher* Dr. Normastura Abd. Rahman
Dr. Zafarina Zainuddin

4. Tajuk Projek: *Title of Project* A study on the *GJB3* and *GJB6* gene mutations among Malays with non-syndromic hearing loss

5. Ringkasan Penilaian/*Summary of Assessment*:	Tidak Mencukupi *Inadequate* 1	2	Boleh Diterima *Acceptable* 3	Sangat Baik *Very Good* 4	5
i) Pencapaian objektif projek: *Achievement of project objectives*	☐	☐	☐	☑	☐
ii) Kualiti output: *Quality of outputs*	☐	☐	☑	☐	☐
iii) Kualiti impak: *Quality of impacts*	☐	☐	☑	☐	☐
iv) Pemindahan teknologi/potensi pengkomersialan: *Technology transfer/commercialization potential*	☐	☐	☑	☐	☐
v) Kualiti dan usahasama : *Quality and intensity of collaboration*	☐	☐	☑	☐	☐
vi) Penilaian kepentingan secara keseluruhan: *Overall assessment of benefits*	☐	☐	☑	☐	☐

6. Abstrak Penyelidikan
(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris.** Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

Abstract of Research
(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English).
This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

- rujuk lampiran -

7. Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.
[Sila gunakan kertas berasingan]
Applicant are required to prepare a Comprehensive Technical Report explaning the project.
(This report must be appended separately)

- rujuk lampiran -

Senaraikan kata kunci yang mencerminkan penyelidikan anda:
List the key words that reflects your research:

Bahasa Malaysia	*Bahasa Inggeris*
Kecacatan pendengaran	Hearing loss
Melayu	Malay
Mutasi gen *GJB2* dan *GJB6*	*GJB2* and *GJB6* gene mutations

8. Output dan Faedah Projek
Output and Benefits of Project

(a) * **Penerbitan Jurnal**
Publication of Journals
(Sila nyatakan jenis, tajuk, pengarang/editor, tahun terbitan dan di mana telah diterbit/diserahkan)
(State type, title, author/editor, publication year and where it has been published/submitted)

Jenis: The Journal of Laryngology and Otology

Tajuk: Screening for gap junction protein beta-2 gene mutations in Malays with autosomal recessive, non-syndromic hearing loss, using denaturing high performance liquid chromatography

Pengarang: Siti Aishah Zainal, Mohd. Khairi Md. Daud, Normastura Abd. Rahman, Zafarina Zainuddin dan Zilfalil bin Alwi

Tahun Terbitan: 2008 , Diterbitkan: United Kingdom

(b) **Faedah-faedah lain seperti perkembangan produk, pengkomersialan produk/pendaftaran paten atau impak kepada dasar dan masyarakat.**
State other benefits such as product development, product commercialisation/patent registration or impact on source and society.

1. Ibubapa yang mempunyai anak yang mengalami kecacatan pendengaran boleh bersedia dari awal daripada segi pendidikan kepada anak yang bakal lahir yang mungkin juga mengalami kecacatan pendengaran.

* Sila berikan salinan/*Kindly provide copies*

(c) **Latihan Sumber Manusia**
Training in Human Resources

i) Pelajar Sarjana: Siti Aishah Zainal
Graduates Students
(Perincikan nama, ijazah dan status)
(Provide names, degrees and status)

Ijazah : Ijazah Sarjana Sains (Genetik Manusia)

Status: Tahun3, Semester 3

ii) Lain-lain:
Others

9. Peralatan yang Telah Dibeli:
Equipment that has been purchased

Tiada

Tandatangan Penyelidik
Signature of Researcher

7/12/09

Tarikh
Date

Komen Jawatankuasa Penyelidikan Pusat Pengajian/Pusat
Comments by the Research Committees of Schools/Centres

This project have achieved all three objectives.
The output of the research project is very good with one international publication in The Journal of Laryngology and Otology beside two paper presentations.

The project has also produced one Face (Kenan article)

Overall performance of the project is good.

PROFESSOR AHMAD SUKARI HALIM
Chairman of Research Committee
School of Medical Sciences
Health Campus
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan.

TANDATANGAN PENGERUSI
JAWATANKUASA PENYELIDIKAN
PUSAT PENGAJIAN/PUSAT
Signature of Chairman
[Research Committee of School/Centre]

7/1/2010
Tarikh
Date

Abstrak penyelidikan

Hearing loss is the most common sensory disorder in human. Previous journals reported that *GJB2* gene is the most important gene involved in non-syndromic hearing loss; and *GJB6* gene is the second most which contributed to. This study is to screen mutations in *GJB2* and *GJB6* gene among Malay patients with non-syndromic hearing loss. Ninety one patients and equal number of normal hearing subjects were recruited for this study after getting informed consent. DNA samples were collected using sterile buccal swab. After DNA extraction, genomic DNA was amplified then screened using dHPLC technique. Certain samples were sequenced to confirm the type of mutation. The result showed that only a few samples having mutations in *GJB2* gene, while no sample having mutation of *GJB6* gene.

Kecacatan pendengaran adalah kecacatan yang paling banyak pada manusia. Banyak jurnal melaporkan bahawa gen *GJB2* adalah gen yang paling penting yang terlibat dalam kecacatan pendengaran tiada sindrom dan gen *GJB6* adalah yang kedua paling banyak terlibat. Penyelidikan ini adalah untuk melihat mutasi pada gen *GJB2* dan *GJB6* di antara pesakit Melayu dengan kecacatan pendengaran tiada sindrom. Sembilan puluh satu pesakit kecacatan pendengaran dan subjek kawalan direkrut untuk kajian ini selepas mendapatkan kelulusan. Sampel DNA diambil dengan menggunakan putik kapas yang steril. Setelah pengekstrakan DNA, DNA genomik diperbanyakkann kemudian disaring dengan menggunakan teknik dHPLC. Beberapa sampel dilakukan penjujukan DNA untuk mengesahkan jenis mutasi. Keputusan kajian menunjukkan bahawa hanya beberapa sampel mempunyai mutasi pada gen *GJB2*, manakala tidak ada sampel mempunyai mutasi gen *GJB6*.

Technical report

This study was approved by Research and Ethics committee, School of Medical Sciences, Universiti Sains Malaysia. 91 Kelantanese Malay patients were recruited diagnosed to have non-syndromic hearing loss (NSHL). The subjects are from Sekolah Kebangsaan Pendidikan Khas, Pasir Mas, Sekolah Menengah Kebangsaan Ismail Petra (Bahagian Pendidikan Khas), Kota Bharu, Kelantan and Audio Clinic, Hospital Universiti Sains Malaysia (HUSM).

After getting the informed consent, buccal swab samples were collected by rubbing the inner cheek of patient and control groups using sterile cotton swab. The swab was taken to Human Genome Centre for analysis.

For DNA extraction, commercial kit, GeneAll (Soeul, South Korea) was used in order to get genomic DNA. To confirm the presence of genomic DNA and its concentration, the samples were run on agarose gel electrophoresis and check the purity and optical density (O.D) using NanoQuant machine. Then, all samples underwent polymerase chain reaction (PCR) using primer pairs as described by Zelante *et al.* (1997) for *GJB2* gene and del Castillo *et al.* (2002) for *GJB6* gene, followed by electrophoresis on agarose gel to see the PCR products. After that, all samples were screened using denaturing high performance liquid chromatography (dHPLC). The samples with heteroduplex peaks were then sequenced to identify the type of mutation. Results were analyzed using statistical analysis.

References

1. del Castillo I, Villamar M, Moreno-Pelayo MA, del Castillo FJ, Alvarez A, Tellería D et al. A deletion involving the connexin 30 gene in nonsyndromic hearing impairment. *N Engl J Med* 2002;**346**:243-249

2. Zelante L, Gasparini P, Estivill X, Melchionda S, D'Agruma L, Govea N, Milá M, Monica MD, Lutfi J, Shohat M, Mansfield E, Delgrosso, Rappaport E, Surrey S, Fortina P. Connexin26 mutations associated with the most common form of non-syndromic neurosensory autosomal recessive deafness (DFNB1) in Mediterraneans. *Hum Mol Genet* 1997;**9**:1605-1609

BORANG LAPORAN HASIL PENYELIDIKAN
PPSP

Tajuk geran: A study on the *GJB3* and *GJB6* gene mutations among Malays with non-syndromic hearing loss
Penyelidik: Dr. Mohd. Khairi Md. Daud
Jenis geran: Jangka Pendek
Tempoh geran: 2 tahun

Jenis laporan: Laporan Kemajuan [ ] Alatan di beli [ ] Ya:nyatakan.....

Laporan Akhir*: [/] [/] Tidak

BJEKTIF SPESIFIK KAJIAN (sama t dalam proposal asal)	**SECARA RINGKAS TERANGKAN PENCAPAIAN/HASIL**	**OBJEKTIF TERCAPAI ATAU TIDAK**
1. To identify the *GJB3* and *GJB6* gene mutations in Malay populations in Malay patients with non-syndromic hearing loss and normal hearing	*GJB6* gene mutation is identified. *GJB3* gene is not identified because the gene had change with other important gene, *GJB2* gene. *GJB2* gene mutations are identified	Achieved
2. To determine the association between the *GJB3* and *GJB6* gene mutations and non-syndromic hearing loss	*GJB6* and *GJB2* gene mutations - determined	Achieved
3. To determine the association between the *GJB3* and *GJB6* gene mutations with the clinical phenotype	*GJB6* and *GJB2* gene mutations - determined	Achieved
4.		

- *Laporan Akhir perlu disertakan salinan manuskrip dan surat yang dihantar kepada mana-mana jurnal untuk penerbitan.*

Nama Penyelidik Utama (PI): Dr. Mohd. Khairi Md. Daud t.t.:
Tarikh: 7/12/09

The Journal of Laryngology & Otology (2008), 122, 1284–1288.
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doi:10.1017/S0022215108002041

Main Article

Screening for gap junction protein beta-2 gene mutations in Malays with autosomal recessive, non-syndromic hearing loss, using denaturing high performance liquid chromatography

Z Siti Aishah, M D Mohd Khairi*, A R Normastura†, Z Zafarina‡, B A Zilfalil

Abstract

Objective: To determine the frequency and type of gap junction protein beta-2 gene mutations in Malay patients with autosomal recessive, non-syndromic hearing loss.

Methods: A total of 33 Malay patients with autosomal recessive, non-syndromic hearing loss were screened for mutations in the Cx26 coding region. Deoxyribonucleic acid was extracted from buccal swab samples and subjected to polymerase chain reaction. Slow-reannealing was performed, followed by screening using denaturing high performance liquid chromatography.

Results: Eight of the samples (24.2 per cent) showed heterozygous peaks, and further sequencing of these samples revealed four patients (50.0 per cent) with the W24X mutation, two (25.0 per cent) with the V37I mutation and another two (25.0 per cent) with the G4D mutation.

Conclusions: Analysis of buccal swab samples by denaturing high performance liquid chromatography is noninvasive and suitable for rapid and reliable screening of gap junction protein beta-2 gene mutations in patients with autosomal recessive, non-syndromic hearing loss. Malay patients with autosomal recessive, non-syndromic hearing loss have different kinds of gap junction protein beta-2 gene mutations which are rarely found in other populations.

Key words: GJB2 Protein, Human; Sensorineural Hearing Loss; Congenital; Malaysia; Human Genetics

Introduction

Hearing loss is the most common congenital sensory deficit in humans. Roughly one to three children in a thousand are born with hearing impairment.[1–5] The occurrence of hearing loss is considerably greater in certain sub-populations; for example, it is present in one to five in 100 neonatal intensive care patients and infants selected from at-risk registers.[3,6–11] Hearing loss can be caused by environmental factors as well as genetic factors. Genetic causes represent 50–70 per cent of hearing loss, with autosomal recessive inheritance representing approximately 80 per cent of this total.[12] It is believed that more than 100 genes may be involved in hearing impairment. Several of these genes have been identified, one of which is the gap junction protein beta-2 gene. The identification of this deafness gene has facilitated understanding of the molecular process of hearing, and it offers prospects for deoxyribonucleic acid (DNA) testing.

Mutations in the gap junction protein beta-2 gene (also know as the connexin 26 gene) are responsible for half of the cases of autosomal recessive, non-syndromic hearing loss. The 35delG mutation of this gene has been reported to be common in several countries.[13] However, in non-white populations, the 35delG mutation is either absent or very rare, with other common mutations prevailing, such as the 235delC mutation in the Japanese,[14] the V37I mutation in Malaysians[15] and the 167delT mutation in Ashkenazi Jews.[16]

The increasing demand for gap junction protein beta-2 gene mutation detection warrants the need for a rapid and accurate method of screening for these mutations. This study was undertaken in order (1) to investigate the types and frequencies of gap junction protein beta-2 gene mutations in Malay patients with autosomal recessive, non-syndromic hearing loss, and (2) to assess the effectiveness of buccal smears and denaturing high

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Accepted for publication: 3 January 2008. First published online 20 March 2008.